Topic 10 – Angiogenesis, microcirculation, growth factors, progenitor cells – B

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0221

MicroRNA-21 coordinates human multipotent cardiovascular progenitors therapeutic potential and post-ischemic revascularization

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Published clinical trials in patients with ischemic diseases show limited benefit of adult stem cell-based therapy, likely due to their restricted plasticity and commitment towards vascular cell lineage. Here, we have uncovered the potent regenerative ability of MesP1/SSEA-1-expressing cardiovascular progenitors enriched from human embryonic stem cells (hESC). Injection of only 10^4 hESC-derived SSEA-1$^+$/MesP1$^+$ cells, or their progeny obtained after treatment with VEGF-A or PDGF-BB, was effective enough to enhance post-ischemic revascularization in immunodeficient mice with critical limb ischemia (CLI). However, the rate of incorporation of hESC-derived SSEA-1$^+$/MesP1$^+$ cells and their derivatives in ischemic tissues was modest. Alternatively, these cells possessed a unique miR-21 signature that inhibited PTEN thereby activating HIF-1α and the systemic release of VEGF-A. Targeting Dicer or miR-21 limited cell survival *in vivo* and inhibited their pro-angiogenic capacities both in the Matrigel model and in mice with CLI. Interestingly, we observed an impaired post-ischemic angiogenesis in miR-21 deficient mice suggesting an unrestricted role of miR-21 in this regenerative environment. Notably, amongst the inflammatory cell population, miR-21 was highly expressed in circulating and infiltrated monocytes where it targeted PTEN/HIF-1α/VEGF-A signaling. As a result, miR-21 deficient mice displayed an impaired number of infiltrated monocytes and a defective angiogenic phenotype that could be partially restored by retransplantation of bone marrow-derived cells from wild-type littermates. Hence, hESC-derived SSEA-1$^+$/MesP1$^+$ cells progenitor cells are powerful key integrators of therapeutic angiogenesis in ischemic milieu and miR-21 is instrumental in this process as well as in the orchestration of post-ischemic vessel growth.

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Proepicardial prokineticin receptor –1 (PKR1) as a developmental link between heart and kidney

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Background: Prokineticin receptor-1 (PKR1), signals play critical roles in heart and kidney functions. In particular, the systemic mutation of this receptor results in thinning of the myocardium and hypoplastic kidney. However, the molecular and cellular mechanisms controlled by PKR1 signaling in this process are unclear.

Methods and Results: Here, we analyze a tissue-restricted mutations of the PKR1 gene in the proepicardial lineages (Gata5 and Wt1), and we show that PKR1 signaling in the proepicardium and its derivatives is required for proper cardiac and renal morphogenesis. Neonatal mutant mice display impaired proliferation of epicardial-derived cells in their heart and kidneys. Moreover, we detect defective coronary and renal arteriogenesis associated with PKR1 deficiency. Epicardial development is dramatically impaired in mutant mice, including failed expansion of the subepicardial space, blunted invasion of the myocardium, and impaired differentiation of epicardium-derived cells into coronary endothelial and smooth muscle cells. Abnormal mitochondria, lipid accumulation in mutant cardiomyocytes leads to lower contractile response to dobutamine. Impaired proliferation was observed in both Gata5 and WT1 but apoptosis was observed only WT1 lieage. Adult mutant hearts had abnormal rhythmicity and impaired systolic functions. Hypoplastic kidneys at the neonatal mutants were accompanied with deficient glomerular angiogenesis. Outgrown cell from kidney explants had a defective vasculogenic cell differentiation. Atrophy and dilated glomerular structure, abnormal mitochondria, lipid deposition and apoptosis were observed in the adult mutant kidney.

Conclusions: Our findings provide a mechanistic insight into the roles of PKR1 signaling in heart and kidney disorders controlling the maturation of epicardial-derived cell and differentiation in a cell autonomous fashion and affecting cellular communications in a paracrine fashion. Our mouse models recapitulate the complex human heart-kidney disorders.

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Vascular remodeling of the endocardium following cardiac infarction occured by arteriogenesis and angiogenesis

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Coronary vasculature is required to maintain cardiomyocyte survival, via delivery of oxygen and nutrients, and consequently myocardial architecture and cardiac function. Ischemic heart disease following myocardial infarction causes irreversible cell loss and scaring and is a major cause of morbidity and mortality. Revascularization of injured, ischemic and regenerating organs is essential to restore organ function and requires the formation of new vessels by the mechanisms of vasculogenesis, angiogenesis or arteriogenesis. With the objective of studying vascular remodeling during myocardial infarction (MI), we have performed permanent left coronary ligation on *Connexin40-GFP* (*Cx40$^{GFP/+}$*) mice. *Cx40* encodes a gap junction protein and is expressed in endothelial cells of large vessels. In the heart, *Cx40-GFP* expression is detected in coronary arteries but not in veins, capillaries or endocardium. After two weeks of ligation, MI was detected in left ventricle by echocardiography and anatomical examination of these hearts revealed the presence of an extensive network of GFP-positive vasculature within the infarct area. These vessels follow a tortuous route in the remaining ventricular wall and some communicate with the left ventricular lumen forming a crater covered with GFP and VEGF-R2 positive endothelial cells at the endocardial surface. To determine whether these vessels result from neo-vascularization or coronary artery remodeling, we carried out genetic lineage tracing of coronary endothelial cells using an inducible *Cx40-cre* allele. Our results show that GFP positive endothelial cells forming the endocardial carters are not always derived from pre-existing coronary arteries, suggesting that endocardium may also contribute to the generation of new vessels during vascular remodeling in the adult heart by angiogenesis.

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Non-peptidic prokineticin receptor 1 agonist as a novel cardioprotective therapeutic

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Objective: Prokineticins are potent angiogenic peptides that bind to two G protein-coupled receptors to initiate their biological effects. We previously

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